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MADISON 6

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Dear Josh:

The system with which Baldwin is working uses my phage 80 as the transducing phage. The donor strains are phage pattern 80/81. A number of these (14) have been tested and all will act as donors. The characters transferred are penicillinase production and bythromycin resistance. The acceptor strains are antibiotic sensitivies of various phage patterns in phage Group 1, e.g. propagating strains for phage 29, phage 42B and phage 52, and some other strains of pattern 29/79/80 and 29/52A/80. The strain accepting is not necessarily sensitive to phage 80. (but does fall into phage Group 1.)

The phage preps are made by lysis, are filtered and contain 10^{10} - 10^{11} particles/ml.

Transductions are made by giving the acceptors 18 - 24 hrs. on Difco brainheart salt slants. The cells are then washed in Difco nutrient broth resuspended in 2 ml brain heart broth(no added NaCl) For every 10 cells 2 X 10 phage is added. The mixture is shaken at 37° for 1 hr. Spun twice to remove non-adsorbed phage -- resuspended in 1 ml broth. 0.05 -0.1 ml aliquots are plated on brain heart infusion afar + 0.12 pen.

The penicillinase producing colonies can be recognized by satellulum fisia of the penicillin sensitives around them. The Em# by colony formation on em containing medium (? Concentration) They semm to have Wasked chiefly with penicillinase transduction and also with the Ema character which is not accepted by all the strains that accept pen'ase. There is no phenotypic lag. with these 2 characters. No double transductions have been observed. There was no success with attempts to transfer Smallor Mann+.

Circa $50/10^8$ cells are transduced. The phage sensitivity pattern of the transduced strain usually remains unaltered and the establishment of lyso genicity does not seem to be an essential condition of the transduction. What is considered essential is that the transductions are made on this brain heart medium in which lysis of the acceptor strain by the transducing phage is inhibited. If transductions are attempted in a medium in which most of the cells are lysed, no transfers can be detected.

There is no evidence yet that transduced strains have acted as fresh donors. But this question has not yet been fully examined.

All this semms quite exciting and on present evidence should be acceptable. I have examined some of the acceptor strains and could never find any evidence of spontaneous mutation to penicillinase production.

When I get home I propose to try the system and will let you know what

CC: Buth Langues phage 53. Nearly all the stapho are bal+

Phyllis